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Anti-proliferate Activity and 5α-reductase Inhibitors of Chiral Macrocyclic (Nα-di-nicotinoyl)[L-phenylalaninyl-L-leucinyl]Pentapeptide Candidates Against LNCaP and PC-3 Prostate Cancer Cell Lines

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A series of macrocyclic derivatives **2-6** were prepared using N^{α}-dinicotinoyl-bis[L-phenylalaninyl-L-leucyl]hydrazide **1** and cyclo pentapeptide hydrazide **2** as starting materials. The hydrazide **1** was cyclized with diaminoalkanes to macrocyclic hezaazahexaamide derivatives **3a-c**, respectively. Finally, compound**2** was condensed with 3,5-diacetylpyridine gave the corresponding macrocyclic Schiff base **4**. Finally, condensation of **2** with substituted aromatic or heterocyclic aldehyde derivatives gave the corresponding Schiff base derivatives **5a-f** and **6a-c**, respectively. The synthesized compounds were screened as 5α-reductase inhibitors and anti-proliferative activity against prostate cancer cell lines.

Keywords: Macrocyclic pentapeptide, Schiff bases, 5α-reductase inhibitors and anti-proliferate activities.

Introduction

Peptides are formed from the chemical reaction between two or more amino acids by different methods[1] and the covalent chemical bonds are formed when the carboxyl group of one amino acid reacts with the amino group of another. Interestingly, some individual amino acids exemplified by valine, leucine, iso-leucine, and phenylalanine were reported to have biological and pharmacological properties in different experimental models[2]. In previous work, we have found that certain substituted heterocyclic compounds show antimicrobial[3], anti-viral[4], and reductase inhibitors[5]. On the other hand, some heterocyclic pyridine derivatives have promising biological[6] and anticancer activity[7]. Recently, in our previous work, some of peptidopyridine derivatives have been synthesized[8,9] and tested as pharmacological activity[10], analgesic and anti-inflammatory[11], antimicrobial[12,13], antiparkinsonian[14], anticancer[15,16] agents. In view of these observations and in continuation of our previous work in pyridine and peptide chemistry, we synthesized some new macrocyclic pyridine pentapeptide compounds and screened as 5α-reductase inhibitors and anti-proliferative activity against prostate cancer cell lines.

Materials and methods

Melting points were determined in open glass capillary tubes with an Electro Thermal Digital melting point apparatus (model: IA9100) and are uncorrected. Elemental microanalysis for carbon, hydrogen and nitrogen (Microanalytical Unit, NRC) was found within the acceptable limits of the calculated values. Infrared spectra (KBr) were recorded on a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer. Proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C NMR) spectra were run in (DMSO-d_6) on Jeol 500 MHz instruments "500 MHz to ^{1}HNMR and 125 MHz to ^{13}CNMR". Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer, using the electron impact technique (EI). Analytical thin layer chromatography

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(TLC) was performed on silica gel aluminum sheets, $60F_{254}$ (E. Merck).

Synthesis of cyclo-(N^{α}-dinicotinoyl)-bis[L-phenylalaninyl-L-leucyl]aliphatic diamines (3a-c)

To a stirred solution of hydrazide **(1)**[8,17,18] (1 mmol) a mixture of 5N HCl (2 ml), AcOH (3 ml) and H_2O (10 ml) at -5°Cfor 15 min, sodium nitrite (0.2 g, 2 ml water) was added. The reaction mixture was stirred for 0.5 h, then extracted with cold dichloromethane (-5°C), which was added to aliphatic diamine, namely, 1,3-propanediamine, 1,4-butanediamine or 1,6-hexanediamine (1 mmol, 10 mL dichloromethane) with stirring for 3 h at -5°C, then at room temperature for 20 h. The mixture product washed with water, 0.5 N hydrochloric acid, water and dried over anhydrous calcium chloride. The solvent was evaporated to dryness, the crude residue were purified by preparative chromatography on silica gel by using chloroform/ethanol (9:1, *v/v*) as eluent to give the corresponding macrocyclic trtrapeptide derivatives **3a-c**,respectively.

3a: Yield 65%; m.p. 206-208°C. $[\alpha]_D^{25}$ = -106 (*c* = 0.5, DMF). IR (film): *v* = 3448-3345 (NH), 1654, 1534, 1254 (3C=O, amides) cm^{-1}. ^1H NMR:δ = 0.88-0.95 (m, 12H, $4CH_3$), 1.72-1.80 (m, 6H, $3CH_2$), 2.22-2.36 (m, 2H, 2CH), 3.30 (t, 4H, $2CH_2$), 3.44 (d, 4H, $2CH_2$), 4.25-4.32 (m, 2H, 2CH), 4.68-4.72 (m, 2H, 2CH), 6.95-7.75 (m, 10H, Ar-H), 8.36 s, 9.05 (s, 3H, pyr-H), 8.66, 8.86, 9.20 (3s, 6H, 6NH, D_2Oexchangeable). ^{13}C NMR: δ = 18.90, 19.40, 24.00, 32.65, 38.80, 39.40, 42.18, 52.90, 54.24, 125.80, 127.50, 128.32, 131.50, 138.75, 141.12, 152.35, 166.72, 169.85, 172.96 (40 C). MS (EI, 70 eV): *m/z* (%) = 726 (13) $[M]^+$. $C_{40}H_{51}N_7O_6$ (725.87): Calcd. C 66.19, H 7.08, N 13.51; found C 66.05, H 7.00, N 13.40.

3b: Yield 74%; m.p. 196-198°C. $[\alpha]_D^{25}$ = -118 (*c* = 0.5, DMF). IR (film): *v* = 3424-3365 (NH), 1658, 1536, 1240 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.86-0.95 (m, 12H, 4 CH_3), 1.28-1.36 (m, 4H, 2 CH_2), 1.40-1.45 (m, 4H, 2 CH_2), 1.68-1.74 (m, 4H, 2 CH_2), 2.26-2.34 (m, 2H, 2CH), 3.36 (d, 4H, $2CH_2$), 4.15-4.24 (m, 4H, 4 CH), 6.98-7.54 (m, 10H, 2Ph-H), 8.48, 9.05 (s, 3H, pyr-H), 8.80, 8.92, 9.16 (3s, 6H, 6NH, D_2Oexchangeable).^{13}C NMR: δ = 19.20, 19.45, 23.95, 28.82, 32.68, 39.42, 42.24, 52.86, 54.36, 125.82, 127.55, 128.36, 131.65, 138.74, 141.18, 152.34, 166.75, 169.80, 172.84 (41C). MS (EI, 70 eV): *m/z* (%) = 740 (13) $[M]^+$. $C_{41}H_{53}N_7O_6$ (739.90): Calcd. C 66.55, H 7.22, N 13.25; found C 66.45, H 7.16, N 13.22.

3c: Yield 70%; m.p. 232-234°C. $[\alpha]_D^{25}$ = -98 (*c* = 0.5, DMF). IR (film): *v* = 3410-3355 (NH), 1660, 1534, 1245 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.86-0.92 (m, 12H, 4 CH_3), 1.24-1.26 (m, 4H, 2 CH_2), 1.32-1.35 (m, 4H, $2CH_2$), 1.42-1.46 (m, 4H, 2 CH_2), 1.62-1.70 (m, 4H, 2 CH_2), 2.26-2.35 (m, 2H, 2CH), 3.44 (d, 4H, $2CH_2$), 4.18-4.24 (m, 4H, 4 CH), 7.00-7.65 (m, 10H, 2Ph-H), 8.42, 9.08 (2s, 3H, pyridyl-H), 8.75, 8.93, 9.16 (3s, 6H, 6NH, D_2Oexchangeable). ^{13}C NMR: δ = 18.95, 19.32, 23.90, 27.82, 32.68, 40.56, 39.48, 42.56, 52.80, 54.34, 125.80, 127.58, 128.34, 138.75, 131.64, 141.24, 152.35, 166.74, 168.98, 172.80 (43C). MS (EI, 70 eV): *m/z* (%) = 768 (16) $[M]^+$. $C_{43}H_{57}N_7O_6$ (767.95): Calcd. C 67.25, H 7.48, N 12.77; found C 67.14, H 7.41, N 12.68.

Synthesis of compound4

A mixture of 3,5-bis-hydrazide **1** (1 mmol) and 3,5-diacetylpyridine(0.163 g, 1 mmol) in AcOH (25 mL) was refluxed for 4 h. The reaction mixture was poured into ice water, the obtained solid was collected by filtration, washed with water and crystallized from AcOH/H_2O to give bicyclic product **4**.Yield 70%; m.p. 248-250°C. $[\alpha]_D^{25}$ = -98 (*c* = 0.5, DMF). IR (film): *v* = 3430-3355 (NH), 1654, 1534, 1252 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.90-0.96 (m, 18H, $6CH_3$), 1.68-1.74 (m, 4H, $2CH_2$), 2.22-2.30 (m, 2H, 2CH), 3.44 (d, 4H, $2CH_2$), 4.20-4.30 (m, 2H, 2CH), 4.65-4.75 (m, 2H, 2CH), 6.96-7.70 (m, 10H, 2Ph-H), 8.20-8.25 (m, 3H, pyr-H), 8.46, 9.08 (2s, 3H, pyr-H), 8.75, 8.85, 9.16 (3s, 6H, 6NH, D_2Oexchangeable). ^{13}C NMR: δ = 13.55, 17.96, 18.45, 23.72, 40.70, 42.42, 52.62, 53.18, 124.28, 128.44, 129.45, 138.80, 155.56, 126.12, 131.74, 135.88, 140.52, 152.45, 154.55, 163.85, 169.35, 178.55 (46 C). MS (EI, 70 eV): *m/z* (%) = 842 (34) $[M-1]^+$. $C_{46}H_{54}N_{10}O_6$ (842.98): Calcd. C 65.54, H 6.46, N 16.62; found C 65.40, H 6.38, N 16.50.

Synthesis of Schiff base derivatives 5a-cand **6a-c**

A mixture of **2** (1 mmol) and substituted aromatic aldehydes "benzaldehyde, *p*-methyl-, or*p*-methoxybenzaldehyde" or heterocyclic aldehydes, "2-, 3-, or 4-pyridinecarbaldehydes" (1 mmol) in absolute ethanol (25 mL) in the presence of a mixture of TEA/DEA (4 ml, 2:2) was refluxed for 4-6 h. The solvent was concentrated to dryness and the obtained residue was solidified with ether, filtered off, washed with ether and crystallized from the proper solvents to afford pentapeptide hydrazone derivatives **5a-c**and **6a-c**,respectively.

5a: Yield 65%; m.p. 210-212°C. $[\alpha]_D^{25}$ = -118 (*c* = 0.5, DMF). IR (film): *v* = 3425-3356 (NH), 1655, 1533, 1234 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.86-0.92 (m, 12H, $4CH_3$), 1.28-1.40 (m, 4H, $2CH_2$), 1.55-1.65 (m, 4H, 2 CH_2), 2.22-2.30 (m, 2H, 2CH), 3.15-3.25 (m, 2H, CH_2), 3.45 (d, 4H, $2CH_2$), 4.00-4.10 (m, 4H, 4CH), 4.42-4.46 (m, 1H, CH), 6.96-7.60 (m, 16H, 3Ph-H + CH=N),8.38, 9.02 (2s, 3H, pyr-H), 8.56, 8.75, 8.86, 9.25 (3s, 7H, 7 NH, D_2Oexchangeable); ^{13}C NMR: δ = 17.75, 18.12, 28.32, 30.36, 38.05, 23.75, 40.92, 41.74, 52.25, 52.85, 58.45, 124.35, 128.35, 128.65, 129.15, 129.45, 131.05, 133.55, 138.71, 142.75, 131.44, 140.14, 152.22, 163.80, 169.18, 170.63, 176.74 (49C). MS (EI, 70 eV): *m/z* (%) = 886 (16) $[M]^+$. $C_{49}H_{59}N_9O_7$ (886.04): Calcd. C 66.42, H 6.71, N 14.23; found C 66.28, H 6.58, N 14.12.

5b: Yield 70%; m.p. 232-234°C. $[\alpha]_D^{25}$ = - 98 (*c* = 0.5, DMF). IR (film): *v* = 3455-3325 (NH), 1652, 1531, 1233 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.90-0.95 (m, 12H, $4CH_3$), 1.22-1.40 (m, 4H, $2CH_2$), 1.56-1.68 (m, 4H, 2 CH_2), 2.18-2.30 (m, 2H, 2CH), 2.40 (s, 3H, CH_3), 3.15-3.25 (m, 2H, CH_2), 3.50 (d, 4H, $2CH_2$), 4.02-4.16 (m, 4H, 4CH), 4.46-4.50 (m, 1H, CH), 7.00-7.64 (m, 15H, 3Ph-H + CH=N),8.50, 9.10 (2s 3H, pyr-H), 8.60, 8.82, 8.90, 9.24 (4s, 7H, 7 NH, D_2Oexchangeable). ^{13}C NMR: δ = 17.84, 18.54, 24.18, 28.37, 30.36, 38.15, 23.74, 40.94, 41.74, 52.30, 52.80, 58.35, 126.00, 127.48, 128.35, 129.06, 129.24, 130.16, 138.90, 140,43, 142.70, 131.46, 140.34, 152.25, 163.80, 169.18, 170.60, 176.90 (50C). MS (EI, 70 eV): *m/z* (%) = 900 (22) $[M]^+$. $C_{50}H_{61}N_9O_7$ (900.07): Calcd. C 66.72, H 6.83, N 14.01; found C 66.64, H 6.70, N 13.94.

5c: Yield 72%; m.p. 244-246°C. $[\alpha]_D^{25}$ = - 106 (*c* = 0.5, DMF). IR (film): *v* = 3418-3350 (NH), 1654, 1532, 1235 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.90-0.96 (m, 12H, $4CH_3$), 1.20-1.34 (m, 4H, $2CH_2$), 1.55-1.60 (m, 4H, 2 CH_2), 2.22-2.34 (m, 2H, 2CH), 3.16-3.24 (m, 2H, CH_2), 3.44 (d, 4H, $2CH_2$), 3.72 (s, 3H, OCH_3), 4.02-4.15 (m, 4H, 4CH), 4.40-4.46 (m, 1H, CH), 6.98-7.60 (m, 15H, 3Ph-H + CH=N),8.44, 9.12 (2s, 3H, pyr-H), 8.56, 8.76, 8.85, 9.24 (4s, 7H, 7 NH, D_2Oexchangeable). ^{13}C NMR: δ = 17.86, 18.42, 28.30, 30.30, 38.00, 23.72, 40.90, 41.80, 52.26, 52.84, 55.74, 58.40, 114.45, 124.45, 125.45, 128.36, 129.34, 129.70, 138.75, 162,46, 142.65, 131.50, 140.20, 152.18, 163.82, 169.20, 170.62, 176.34 (50C). MS (EI, 70 eV): *m/z* (%) = 916 (20) $[M]^+$. $C_{50}H_{61}N_9O_8$ (916.07): Calcd. C 65.56, H 6.71, N 13.76, found C 65.46, H 6.60, N 13.68.

6a: Yield 65%; m.p. 246-248°C. $[\alpha]_D^{25}$ = -104 (*c* = 0.5, DMF). IR (film): *v* = 3410-3340 (NH), 1655, 1535, 1235 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.86-0.95 (m, 12H, $4CH_3$), 1.22-1.34 (m, 4H, $2CH_2$), 1.55-1.70 (m, 4H, 2 CH_2), 2.24-2.32 (m, 2H, 2CH), 3.15-3.26 (m, 2H, CH_2), 3.45 (d, 4H, $2CH_2$), 4.00-4.12 (m, 4H, 4CH), 4.40-4.46 (m, 1H, CH), 7.12-7.29 (m, 10H, 2Ph-H), 7.39-8.20 (m, 5H, pyr-H + CH=N),8.45, 9.12 (2s, 3H, pyr-H), 8.58, 8.74, 8.86, 9.45 (4s, 7H, 7 NH, D_2Oexchangeable). ^{13}C NMR: δ = 17.64, 17.94, 28.38, 30.36, 38.08, 23.86, 40.95, 41.74, 52.35, 52.85, 58.45, 119.34, 125.12, 135.65, 148,65, 152.98, 124.98, 127.60, 128.68, 136.16, 143.82, 131.55, 140.15, 152.15, 165.54, 169.45, 170.76, 175.12 (48C). MS (EI, 70 eV): *m/z* (%) = 886 (35) $[M]^+$. $C_{48}H_{58}N_{10}O_7$ (887.06): Calcd. C 64.99, H 6.59, N 15.79; found C 64.90, H 6.52, N 15.72.

6b: Yield 60%; m.p. 225-227°C. - $[\alpha]_D^{25}$ = 118 (*c* = 0.5, DMF). IR (film): *v* = 3424-3345 (NH), 1652, 1533, 1234 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.90-0.96 (m, 12H, $4CH_3$), 1.24-1.32 (m, 4H, $2CH_2$), 1.52-1.67 (m, 4H, 2 CH_2), 2.25-2.30 (m, 2H, 2CH), 3.18-3.25 (m, 2H, CH_2), 3.48 (d, 4H, $2CH_2$), 4.04-4.10 (m, 4H, 4CH), 4.38-4.45 (m, 1H, CH), 7.15-7.32 (m, 10H, 2Ph-H), 7.54-8.40 (m, 3H, pyr-H), 8.65 (s, 1H, CH=N),8.48, 9.02, 9.14 (3s, 4H, pyr-H), 8.56, 8.78, 8.82, 9.46 (4s, 7H, 7 NH, D_2Oexchangeable). ^{13}C NMR: δ = 17.74, 17.90, 28.50, 30.64, 38.19, 23.85, 40.95, 41.52, 52.76, 52.80, 58.56, 123.08, 129.34, 133.12, 148,75, 150.68, 124.66, 127.74, 128.80, 136.10, 142.72, 131.70, 140.56, 152.26, 165.90, 169.60, 171.00, 175.34 (48C). MS (EI, 70 eV): *m/z* (%) = 887 (16) $[M]^+$. $C_{48}H_{58}N_{10}O_7$ (887.06): Calcd. C 64.99, H 6.59, N 15.79; found C 64.88, H 6.52, N 15.68.

6c: Yield 72%; m.p. 212-214°C. $[\alpha]_D^{25}$ = - 96 (*c* = 0.5, DMF). IR (film): *v* = 3458-3350 (NH), 1652, 1532, 1233 (3 C=O, amides) cm^{-1}. ^1H NMR: δ = 0.88-0.94 (m, 12H, $4CH_3$), 1.20-1.30 (m, 4H, $2CH_2$), 1.55-1.68 (m, 4H, 2 CH_2), 2.23-2.30 (m, 2H, 2CH), 3.22-3.26 (m, 2H, CH_2), 3.44 (d, 4H, $2CH_2$), 4.00-4.12 (m, 4H, 4CH), 4.40-4.46 (m, 1H, CH), 7.10-7.34 (m, 10H, 2Ph-H), 7.86-8.52 (m, 5H, pyr-H + CH=N),8.56, 9.16 (2s, 3H, pyr-H), 8.56, 8.74, 8.80, 9.42 (4s, 7H, 7 NH, D_2Oexchangeable). ^{13}C NMR: δ = 17.60, 17.92, 28.46, 30.50, 38.12, 23.90, 40.96, 41.50, 52.40, 52.56, 58.52, 119.78, 144.00, 149,05, 124.87, 127.68, 128.75, 136.18, 144.65, 131.70, 140.56, 152.26, 165.60, 169.24, 170.56, 175.42 (48C). MS (EI, 70 eV):

m/z (%) = 886 (42) $[M-1]^+$. $C_{48}H_{58}N_{10}O_7$ (887.06): Calcd. C 64.99, H 6.59, N 15.79; found C 64.90, H 6.5o, N 15.70.

Biological Activities

5α-Reductase inhibitors

The experimental method which was used in 5α-reductase inhibitors assay has been adopted from Farghaly *et. al.*,2012[19] (Fig. 1)

Anti-prostate cancer screening anti-androgenic bioassay in human prostate cancer cells

The experimental method which was used in 5α-reductase inhibitors assay has been adopted from Yu*et.al.*, 2018[20].

Results and Discussion

Chemistry

In the present work, some of newly synthesized macrocyclic pentapeptide derivatives **2-6** were obtained by using cyclo penta-peptide hydrazide **2** and N^{α}-dinicotinoyl-bis[L-phenylalaninyl-L-leucyl] hydrazide **1** as starting materials, which were synthesized from 3,5-pyridinedicarbonyl chloride, according to the previous reported procedures[17]. Cyclization of **1** with diaminoalkanes by azide method or with 3,5-diacetylpyridine in refluxing acetic acid afforded the corresponding cyclohexaa-zahexacarboxamide pyridines **3a-c** and bicycle-pyridine derivative **4**. Condensation of hydrazide **2** with substituted aromatic or heterocyclic aldehydes afforded the corresponding Schiff base derivatives **5a-c** and **6a-c**, respectively (Scheme 1).

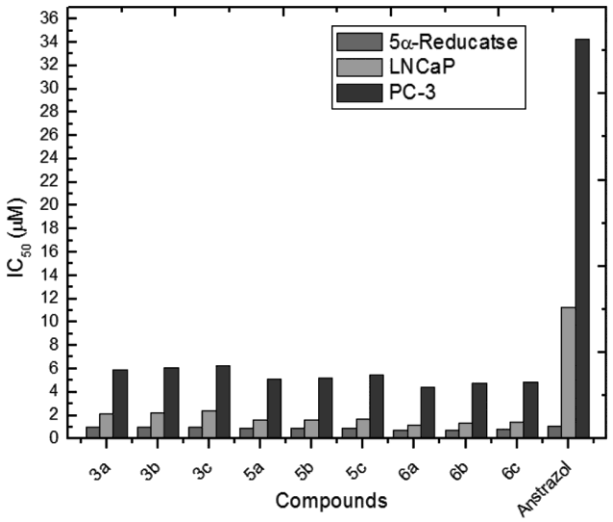
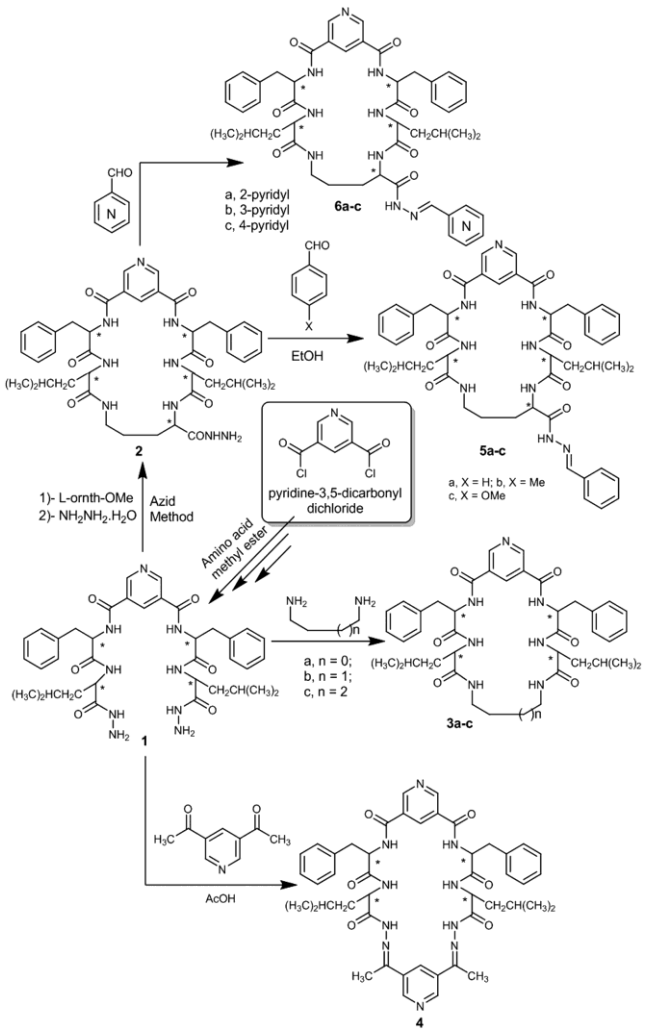


Fig. 1 — 5α-Reductase inhibitors activities and anti-proliferative activity against prostate different cancer cell lines

Biological Screening

A pentapeptide (Ile-Leu-Tyr-Met-Pro; ILYMP) was isolated from the protein hydrolysate of *Cyclina sinensis* was named *Cyclina sinensis* pentapeptide (CSP) and it was found to inhibit DU-145 cell proliferation at a half-maximal inhibitory concentration of 11.25 mM at a 72 h time interval[20]. ILX651 is a pentapeptide has potential for activity across a wide number of solid tumors. ILX651 in vitro cytotoxicity was recently assessed against the NCI panel consisting of 60 human tumor cell lines[21-23]. Cytotoxicy observed across the breadth of tumors tested (melanoma, NSCLC, prostate, breast, colon, CNS, and leukemia) with GI_{50}'s ranging from <10 nM to >1mM. The in vivo antitumor efficacy of ILX651 was assessed against human MX-1 breast tumor



Scheme 1 — Synthetic routes for compounds **2, 3a-c, 4, 5a-c**and **6a-c**

with different dosages, treatment schedules, and administration routes.

5α-Reductase inhibitors

The newly synthesized compounds were screened for their 5α-reductase inhibitors activities in vivo and the obtained results showed potent 5α-reductase inhibitors activities. The descending order of 5α-Reductase inhibitor activities was as follow: **6a, 6b, 6c, 5a, 5b, 5c, 3a, 3b, 3c**.

Anti-proliferate activity against prostate cancer cell lines

The obtained high potent 5α-reductase inhibitors activities encouraged the authors to identify their anti-prostate cancer in vitro using two prostate cell lines namely, LNCaP and PC-3 aiming at reaching a drug that combine both 5α-reductase inhibitors activities and anti-prostate cancer activities. All the tested compounds were screened as anti-tumor activities in two prostate cell lines namely, LNCaP and PC-3. The descending order of anti-tumor activities in two prostate cell lines was as follow: **6a, 6b, 6c, 5a, 5b, 5c, 3a, 3b, 3c.**

Conclusion

All the tested compounds showed potent 5α-reductase inhibitors activities and 5α-reductase inhibitors activities. The descending order of activities was as follow: **6a, 6b, 6c, 5a, 5b, 5c, 3a, 3b, 3c**.

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